IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner: Tsay, Marsha M
Group Art Unit: 1656

In re PATENT APPLICATION of
Applicants : Hosokawa, Kazuya et al.
Serial No. : 10/599,050
Filed : June 29, 2007
For : THROMBIN DERIVATIVES AND MEDICINAL COMPOSITION CONTAINING THE SAME

AMENDMENT

ARGUMENTS IN SUPPORT OF PRE-APPEAL BRIEF REQUEST FOR REVIEW

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
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Dear Sir,

In connection with the Notice of Appeal to the Board of Patent Appeals and Interferences from the Advisory Action dated December 8, 2011 and the Pre-Appeal Brief Request for Review concurrently filed herewith, Applicants hereby submit arguments in support of such Request.

ARGUMENTS

In the Final Rejection of 2011-09-08, under 35 U.S.C. 103(a), claims 1-4, 11-18, 20, 38, 48, 50 & 58-60 were rejected as being unpatentable over Arcone (1999 Biochimica et Biophysica Acta 1451:173-) in view of Morrison (2001 Current Opinion in Chemical Biology 5: 304-) and current practice in protein design (Wells, 1990 Biochemistry 29 (37): 8509-), claims 39, 42-43 & 45-46 rejected over Arcone in view of Morrison, Wells and Veronese (2001 Biomaterials 22: 405-), and claim 47 rejected over Arcone in view of Morrison, Wells, Veronese and Roberts (2002 Advanced Drug Delivery Reviews 54: 459-). The Advisory Action of 2011-12-08 maintained the rejections.

Examiner's arguments were based on the opinion that Arcone teaches a single point mutation at H43 or S205 of thrombin, Morrison teaches combinatorial alanine-scanning where a histidine or serine residue can be replaced by an alanine residue (H→A or S→A, H43A or S205A), Wells teaches a cumulative effect (additivity) of point mutations, and their combination leads to the feature (H43A+S205A) of independent claims 1-2 & 20.

Applicants' Arguments on 2011-11-23 mainly included the following points.

First, Wells only shows that the effects of amino acid substitutions are well cumulative in the specific protein "subtilisin". One of ordinary skill in the art well understands that whether the effects of amino acid substitutions are cumulative or not depends on the protein to be mutated. As indicated in the conclusion of Wells, there are certain exceptional cases for the cumulative effect (additivity) of mutants. Thrombin is just a case where the cumulative effect (additivity) of mutants is absent, as shown in the Examples described in the specification.

Second, H43M disclosed in Arcone still maintains thrombin activity, but S205A or G203A did not show detectable activity. Accordingly, in view of Arcone and Wells, one of

ordinary skill in the art naturally would have selected the combination of positions 205 and 203, rather than the combination of positions 43 and 205. Thus, the effect of the claims of this invention is unexpected and non-obvious to the prior art.

Third, Morrison simply teaches a general method for studying the importance of a non-alanine amino acid by substituting the amino acid with alanine (Abstract), and either can not show an effect of combining the mutations at S205 and H43.

In response, Examiner argued in the Advisory Action of 2011-12-08 as follows.

Regarding the above first point, it should be noted that the Wells was cited as evidence to show that it was known in the art that additive mutagenesis is a tool used in designing functional properties in proteins, in general, but was not cited to note only subtilisin. One of ordinary skill in the art would know that subtilisin was the protein model used to demonstrate additive mutagenesis.

Regarding the above second point, it should be noted that a prior art reference must be considered in its entirety. Since Arcane discloses that H43N dramatically impaired the enzyme activity as well, one of ordinary skill would reasonably select the combination of 43 and 205 since both 43 and 205 are identified as residues in the catalytic triad.

Regarding Applicants' remarks that the effect of this invention is unexpected and non-obvious, it should be noted that the reason or motivation to modify the reference may often suggest what the inventor has done, but for a different purpose or to solve a different problem. In this instance, since single amino acid substitutions in the catalytic triad were identified to disrupt enzyme activity, it would be reasonable for one of ordinary skill to combine the single amino acid substitutions selected from H43, 099 and S205, in order to arrive at the combination of H43

and S205 because there are only three residues at the catalytic triad and it would be reasonable to determine which two residues.

Applicants disagree with these, for at least the reasons set forth.

First, subtilisin is truly a good protein model among various proteins with additive mutagenesis, but thrombin is just a case without additive mutagenesis, i.e., a case where the cumulative effect (additivity) of point mutations is absent, as clearly indicated by the Examples described in the specification and as explained in details in the Response of 2011-06-15 and the in Response of 2011-11-23.

Moreover, Wells mentions in the Conclusion (p. 8515, from the middle of the right column): "***Large deviations*** *from simple additivity can occur when the sites of mutations* ***strongly interact with one another*** *(by making direct contact or* ***indirectly through*** *electrostatic interactions or* ***large structural perturbations****) and/or when* ***both sites function cooperatively*** *(as for* ***the catalytic triad*** *and oxyanion binding site of subtilisin)*" (2nd paragraph). Accordingly, since H43 and S205 are two residues in the catalytic triad of thrombin, they strongly interact with one another and function cooperatively, so that large deviations from simple additivity can occur.

Due to the large deviations from simple additivity, in view of Wells, one of ordinary skill in the art would not have selected the combination of H43 and S205 in the catalytic triad of thrombin to obtain a cumulative effect (additivity) of point mutations, regardless of the fact that H43M maintains the thrombin activity or H43N dramatically impairs the thrombin activity.

Accordingly, Applicants submit that a combination of point mutations at H43 and S205 of thrombin is non-obvious in view of Arcone and Wells.

In addition, Applicants submit that it is also non-obvious to combine Morrison with Arcone and Wells. As noted by Examiner, Morrison discloses that combinatorial alanine-scanning can be used to rapidly identify residues important for protein function, stability and shape (p. 302). Since the residues important for thrombin (protein) function, stability and shape are already known in the prior art (including H43 and S205, see Arcone, for example), in view of Morrison, one of ordinary skill art would not have been motivated to apply alanine-scanning to study thrombin, not mentioning to replace the amino acid residues at specific positions (43 and 205) of thrombin even when Arcone and Wells are purposely combined to obtain the mutation combination of H43+S205.

Furthermore, the fact that H43M maintains thrombin activity but H43N dramatically impairs thrombin activity indicates that the thrombin activity significantly depends on the species of the amino acid at the position. It is also generally known in the art that the effect of a substitution with a specific kind of amino acid residue at a specific position of a protein is difficult to know without an experiment. Hence, in view of the prior art, it is difficult to predict or expect the effect of the single point mutation of H43A to thrombin, not mentioning to predict or expect the effect of the combination of H43A+S205A recited in claim 1 to thrombin.

Accordingly, the combination of the mutations at H43 and S205 as recited in claim 1 is non-obvious over Arcone, Wells and Morrison, and the effect of such combination to thrombin as mentioned in the Response of 2011-06-15, i.e., completely losing thrombin activity but maintaining substrate-binding ability, is unexpected in view of the prior art.

In the case of *Procter & Gamble Co. c. Teva Pharmaceuticals USA, Inc., 566 F.3d 989 (Fed. Cir. 2009)*, the Federal Circuit noted in dicta that even if a *prima facie* case of obviousness had been established, sufficient evidence of unexpected results was introduced to rebut such a showing.

Moreover, according to the case of *Sanofi-Synthelabo v. Apotex, inc., 550 F.3d 1075 (Fed. Cir. 2008)*, a claimed isolated stereoisomer would not have been obvious where the claimed stereoisomer exhibits unexpectedly strong therapeutic advantages over the prior art racemic mixture without the correspondingly expected toxicity, and the resulting properties of the enantiomers separated from the racemic mixture were unpredictable.

The other references cited for rejecting some dependent claims, Veronese and Roberts et al., also fail to teach the above feature of the independent claims 1, 2 & 20.

For at least the above reasons, Applicants submit that claims 1-2 & 20 and claims 3-4, 11-18, 38-39, 42-43, 45-48, 50 & 58-60 dependent therefrom all patently define over the prior art, hence requesting withdrawal of all the rejections under 35 U.S.C. 103(a).

Respectfully submitted,
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